CHAPTER 1. Introduction

There are vast differences in the education of an engineer and a biologist. Many speculate at a chemical engineer’s interest in biology and how the two subjects can possibly associate. This text will help both to provide a clear understanding of the valuable information that can be obtained from the union of the two differing disciplines and to present new insight into growth factor activity. The biologist’s devotion to extensive lab research and the engineer’s constant desire for a mathematical model to manipulate will both be satisfied. They are not in opposition but complement each other and produce a more thorough examination of the subject.

A. Philosophy

The main goal of research in any field is to somehow better the world in which we live. Combining aspects from different disciplines can provide insight via diverse perspectives and also can produce rapid results. The nature of biological research requires controlled experiments that alter parameters in a systematic manner and can cost the researcher valuable time and resources. The amount of actual experimental time may be shortened with the application of engineering modeling techniques. The parameters that are difficult to experimentally modify may now be adjusted with the push of a button on a computer simulated model. The model will allow the researcher to “experiment” with parameters on the computer and get feedback, which can later be used to guide experimental research.

The primary aim of this text is to gain insight on how cellular activation by a growth factor, in the presence of binding proteins, is influenced by proteoglycans. Initial research will be presented, assumptions and hypotheses that were included in the development of the mathematical model will be discussed, and the future enhancements of the model will be explored. There are many potential scenarios for how each component might influence the others. Simple steady state and transient modeling techniques will be presented. Modeling techniques will highlight the contributions made by numerous extracellular parameters on growth factors. Tentative assumptions can be applied to modeling techniques and predictions may aid in the direction of future experiments.
B. Overview

Growth and development in biological systems are modulated by micromolecules, such as growth factors, and macromolecules, like growth factor receptors, growth factor binding proteins and proteoglycans. The binding of a growth factor to the cell surface receptor triggers a signal transduction pathway that can lead to some type of cell stimulation such as cell proliferation, cell differentiation, or cell death. This signaling occurs through reinforcing combinations of low energy force interactions and covalent enzyme-mediated modifications.

The regulation of cell functions, such as cell stimulation by growth factors, has major implications for the pharmaceutical industry. Work investigating how to produce medicinal agents that will ape, substitute or arrest certain cell functions is dependent on knowledge of growth factor activity in its intended environment. Many diseases, including atherosclerosis, cancer and rheumatoid arthritis, are characterized by chronic inflammation are associated with the expression and cellular coordination of the action of growth factors. For example, endothelium damage during balloon angioplasty may augment the production of insulin-like growth factor-I (IGF-I). Alterations in circulating IGF-I and insulin-like growth factor binding proteins (IGFBPs) in diabetic animals are accompanied by modifications of the IGF-I system in the cerebellum and possibly other brain regions. The changes of the IGF-I system may be responsible for specific neuronal losses known to occur in diabetic patients.

Malignant transformation introduces many changes in cultured cells. Alterations in growth parameters and cell behavior can result in continuous division when normal cells cease to divide. Decreased growth factor requirements allow malignant cells to grow in lower initial serum components than normal cells. Transformed cells have lost some hormone and growth factor requirements. Some transformed cells become autostimulated via expression of their own growth factors and corresponding growth factor receptors.

Tumor research could be advanced through increased knowledge of growth factor regulation. At least seven types of proteins participate in the growth control process: growth factors, growth factor binding proteins, proteoglycans, growth factor
receptors, intracellular signal transducers, nuclear transcription factors and cell-cycle control proteins. Mutations in specific molecules from growth control proteins can precede cancer⁵. Mutations changing the structure or expression of growth factors, receptors, signal transducers and transcription factors can lead to dominantly active oncogenes. Oncogenes can originate from genes encoding growth factors, but growth factor receptors are more typically turned into a receptor in a permanent “on” state⁶. IGF-I receptor (IGF-IR) is responsible for mediating IGF-I mitogenic effects and transforming potential of many cells. The overexpression of IGF-IR in a large array of cancers (lung, breast, colon, renal, leukemia, gastric) has been shown⁴. IGF-IR may be responding to circulating IGF-I (endocrine) or to IGF-I locally produced either by neighboring cells (paracrine) or by the cancer cells themselves (autocrine)⁶. With increased comprehension of how the growth factors bind to receptors, tumor growth may be more effectively suppressed.

Chart 1-1 Inspection levels related to growth factors
C. Background Information

Mature animal cells are, in general, specialized to perform one primary function. They contain a specific pattern of receptors that allow themselves to react to each of the distinct chemical signals that issue or accentuate a certain function. The bulk of chemical signals impact their target cells by either changing the properties of the cell, altering conformation of existing proteins, or by initiating the synthesis of new proteins.

Growth control of a cell is only understood in the broadest of terms. Concentrated research is being done to open the narrow field of knowledge on growth control and the related molecules that affect cellular growth control. As with any unknown, beginning with known and simple facts may help to shed light on the subject.

The membrane surrounding a cell separates and protects the cell from the external environment. A cell membrane is dynamic; it is in a constant state of flux in response to intracellular and extracellular changes. The membrane is asymmetrical with a lipid bilayer and membrane proteins. The lipid bilayer constitutes 50% of the plasma membrane and provides the basic structure for the membrane. Due to the high content of lipids in the membrane, water soluble (hydrophilic) molecules can not permeate through the membrane, but lipid soluble (hydrophobic) molecules can. With

![Diagram of membrane proteins](image-url)
lipids playing the structural role for the membrane, proteins have to be the functional operator. Membrane proteins account for 50% of the plasma membrane and link the cytoskeleton of the cell to neighboring cells. Proteins can catalyze various reactions on the cell surface and inside of the cell and act as antennas to receive chemical signals. Membrane proteins can reside on the cell surface attached to fatty acids or carbohydrates or they can traverse the plasma membrane. Transmembrane proteins that pass through the membrane several times function as gatekeepers for certain molecules and allow them to pass through the membrane, (Figure 1-1).

Cells communicate with each other over short and long distances via chemical signals. This communication is vital for the organization of tissues, growth control and division, and other cellular functions. Endocrine signaling, long distance communication between cells, involves hormones which act on target cells distant from their site of synthesis by cells of endocrine organs. The hormones are usually carried in the bloodstream from the release site to the target site. An endocrine cell must pick up a very dilute signal with a sensitive receptor due to the low concentration (less than $10^{-8}$ M) of the signaling molecules. Paracrine signaling involves signaling molecules released from a cell that only affect target cells in close proximity to it. Gap junctions are a
specialized type of paracrine signaling where neighboring cells communicate via a direct connection of the cytoplasm of two cells. Autocrine signaling involves cells that respond to substances that the cells themselves released⁶, (Figure 1-2).

Signaling molecules can be either lipid soluble or water soluble molecules. Steroids, amino acid derivatives, fatty acids, proteins, glycoproteins and many more molecules serve as signals. Steroid and thyroid hormones are hydrophobic molecules which can pass through the membrane after being released by carrier proteins. Hydrophilic molecules compensate for their inability to permeate the plasma membrane by binding to specific receptor proteins on the cell surface, (Figure 1-3). Hydrophilic signaling molecules are generally removed or degraded within minutes of entering the extracellular matrix or bloodstream. Therefore, hydrophilic signaling molecules have to reach the intended receptor with efficiency and are normally used for short duration and short distance communication. Lipid soluble molecules can survive for hours or days in the bloodstream before degradation and have long-lasting responses¹.

**Figure 1-3** Hydrophilic signaling molecules (IGF-1), unable to penetrate the plasma membrane, bind to a cell surface receptor on the target cell. Hydrophobic signaling molecules (steroid and thyroid hormones), able to cross the plasma membrane, are transported to the cell by carrier proteins and bind to an intracellular receptor.

Growth factors fall into the short duration and distance communication category of cell signaling molecules. The function of a growth factor is to serve as a mechanism for coupling a cell to the extracellular environment and thus provide the cell with plasticity to respond appropriately to external changes or to changes in the cell state during different stages of cell growth⁷. They are generally extracellular polypeptide
molecules that bind to a cell surface receptor triggering a signal transduction pathway that can lead to cell proliferation or some specific differentiation responses.

Any protein that binds a specific extracellular signaling molecule (ligand) that induces a cellular response qualifies as a receptor. Receptors for steroid hormones, which diffuse across the plasma membrane, are located within the cell. Receptors for water soluble hormones and polypeptide growth factors are located in the plasma membrane with the ligand binding domain exposed to the external medium and bind the ligand with high affinity ($K_D = 10^{-15} - 10^{-8}$ M).

The receptors for many growth factors (e.g. epidermal growth factor, platelet-derived growth factor, insulin-like growth factor) are receptor tyrosine kinases. Receptor tyrosine kinases are an important class of cell surface receptors whose cytosolic domains have tyrosine-specific protein kinase activity that is activated by growth factor binding. The binding leads to receptor autophosphorylation, which incites particular intracellular transduction pathways, (Figure 1-4).

![Figure 1-4](Image)

**Figure 1-4** Cell-surface receptors for many growth factors are receptor tyrosine kinases. Growth factor binding activates the receptor and creates dimers which autophosphorylate tyrosine residues in the intracellular domain. (Adapted from Lodish et al/1995, Molecular Cell Biology)
A great number of cell surface receptors that bind growth factors are thought to undergo a conformational change when they bind the ligand at the cell exterior. This change leads to the generation of an intracellular signal that alters the behavior of the target cell. For example, the activation of insulin-like growth factor I receptor (IGF-IR) blocks osmotic mediated programmed cell death in neurons.

The number and activity of functional growth factor receptors on the cell surface fluctuates. Up-regulation increases the number of receptors on the cell surface while down-regulation decreases the amount of receptors available for ligand binding. This allows the cell to respond optimally to minute changes in growth factor levels. While small growth factor concentration increases cause typical growth factor induced responses, prolonged exposure of a cell to high concentrations of growth factor usually results in a reduction of functional cell surface receptors. Down-regulation of cell surface receptors can occur through the internalization of the receptor by endocytosis where the receptor is either degraded or stored in intracellular vesicles. Down-regulation can also occur by modification of the activity of the receptor. This does not decrease the number of the receptors found on the surface, it merely modifies the activity so that the receptors cannot bind ligand or can bind ligand, but forms a ligand-receptor complex that is incapable of inducing a normal cellular response, (Figure 1-5).

Experimental procedures with labeled ligands have shown that many signaling molecules enter target cells by receptor-mediated endocytosis. It is plausible then, that
the signaling molecules act directly within the cell. However, most receptor-mediated endocytosis result in the transfer of extracellular molecules to lysosomes. Thus, in order to gain entry into the cytosol, the extracellular signaling molecule must have a distinct mechanism to escape the endocytotic vesicle or lysosome to act directly within the cell. Signaling molecules do not have to penetrate the cell to incite a response. For example, the effects of insulin can be imitated by a specific antibody that binds to insulin receptors on the cell surface but is not endocytosed. Insulin is normally endocytosed by the cell, indicating that insulin itself cannot be the signal. The possibility that the cell surface receptor, which is endocytosed along with the growth factor, acts as the intracellular signal is still open for discussion.

It has been shown that growth factor binding to cell surface receptors does not occur by simple passive diffusion. Intrinsic properties of growth factors can lead to binding to other extracellular molecules prior or in place of binding to a cell surface receptor. Whether extracellular molecules support or encumber growth factor binding to a soluble receptor is an area of contention among researchers and is likely to be a function of the specific growth factor and system. Intracellular signaling is dependent on the ability of the growth factor to interact with the extracellular molecules that can regulate ligand-receptor binding at the appropriate interval and locality. Therefore,

![Diagram](image.png)

Figure 1.6  Schematic illustration depicting the possible role a growth factor binding protein may play in relation to the growth factor. 1. The growth factor binding complex may aid in directing the growth factor transport. 2. The growth factor binding protein may interfere or enhance growth factor binding to the receptor. 3. The growth factor binding protein may shield the growth factor from premature degradation.
growth factor regulation requires concentrated knowledge of binding proteins and other extracellular molecules that may regulate the binding proteins. Binding proteins for growth factors may either be a part of the growth factor and under the same control during synthesis (transforming growth factor-beta (TGF-β))⁶ or be an entirely separate element that is controlled independently from the growth factor (insulin-like growth factor (IGF-I))¹². The growth factor binding protein complex may aid in directing the growth factor transport, interfere or enhance growth factor-receptor binding or shield the growth factor from premature expiration¹³. A binding protein may have only one or all of these functional roles, (Figure 1-6).

Besides acting as structural components of the extracellular matrix and anchoring cells to the matrix, proteoglycans have recently gained respect as secondary regulators of growth factors¹⁴. Binding of the proteoglycan to the growth factor, whether it be to the complex of growth factor/growth factor binding protein or to the growth factor itself, is thought to have an important role in growth factor regulation¹⁵.

Stable binding of the growth factor with extracellular molecules like proteoglycans can alter the concentration and effect of the growth factor by storing the growth factor until needed¹⁶, synergizing with the growth factor¹⁷ and stabilizing the growth factor¹⁸,¹⁹ or increase the life span of the growth factor²⁰. For example, heparin can increase acidic fibroblast growth factor (FGF-1) mitogenic activity¹⁷. While heparin or heparan sulfate proteoglycans may²¹,²² or may not²³ be necessary for bFGF (FGF-2) receptor binding or internalization in vivo, they do increase the affinity of bFGF for the receptor²³. Activity of TGF-β1 can be inhibited by some extracellular proteoglycans²⁴, while other proteoglycans may assist in transporting TGF-β1 to the cell surface receptor.

Proteoglycans are subclass of glycoproteins. Glycoproteins typically contain 1-60% carbohydrate by weight in the form of numerous relatively short (generally less than 15 sugar residues) branched oligosaccharide chains of variable composition²⁵. In contrast, proteoglycans are much larger with 90-95% carbohydrate by weight in the form of many long unbranched glycosaminoglycans (GAG) chains with a strong negative charge¹⁵. Glycosaminoglycans are long linear polymers of a repeating disaccharides, consisting of pairs of sugar acids and amino sugars, and may have residues that are sulfated. Proteoglycans vary from each other in molecular size and
number or type of GAG chains, (Figure 1-7). GAG chains are covalently linked to a core protein with more than one type of GAG chain often found in a particular proteoglycan\textsuperscript{26}. The primary GAG chains found in proteoglycans are chondroitin sulfate, dermatan sulfate, heparan sulfate and keratin sulfate\textsuperscript{25}, (Figure 1-8).

\[ \text{Proteoglycan} \]
\[ \text{Unbranched Glycosaminoglycans} \]
\[ \text{Core Protein} \]

\[ \text{Chondroitin Sulfate} \]
\[ \text{Dermatan Sulfate} \]
\[ \text{Heparan Sulfate} \]
\[ \text{Keratin Sulfate} \]

\[ \text{Figure 1-7} \quad \text{Schematic illustration of a typical proteoglycan. GAG chains are attached to a core protein.} \]

\[ \text{Figure 1-8} \quad \text{Proteoglycans are named according to the structure of their repeating disaccharide. Disaccharide units for each representative GAG are shown.} \]
Proteoglycans are named according to the structure of their principal repeating disaccharide. The organization of GAG chains and proteoglycans in the extracellular matrix (ECM) is unclear. The fine structure of heparan sulfate can differ on identical proteoglycan core proteins and these differences can control fundamental cellular properties such as cell-matrix adhesion. Proteoglycans are detected in nearly all extracellular matrices and some are affixed to the plasma membrane. The core protein of a cell surface proteoglycan (CSPG) traverses the plasma membrane and contains short cytosolic domains as well as lengthy external domains to which a number of GAG chains are attached. CSPG are thought to anchor cells to matrix fibers, (Figure 1-9).

Growth factor activation may incite the synthesis and release of proteoglycans and in turn, proteoglycans may free growth factors in close proximity to the cell and in the circulating bloodstream. TGF-β increases up to 20-fold the expression of chondroitin sulfate and dermatan sulfate proteoglycan regulating level and molecular size of these proteoglycans. Proteoglycans secreted by vascular endothelial cells are capable of curtailing some cell type growth. Endothelial proteoglycans have inhibitory activity for both bFGF binding and bFGF smooth muscle cell proliferation.

While it is worthwhile to isolate particular components of the cell and gain knowledge on how each component works, the sensitive nature of growth factor-
receptor binding requires a more in-depth investigation. Blending the accrued information on each cellular component with knowledge of the response of each component to another component can lead us down exciting avenues of cell manipulation. Proteoglycans may be able to regulate binding proteins. Binding proteins may regulate growth factors, and growth factors can bind to receptors and initiate a cellular response. From this simple model, we can predict that the whole process from inception to completion can be manipulated via one cellular component: the proteoglycan. Of course, cells are known to have redundant pathways of control which may invalidate this simple model but understanding the primary pathway brings us one step closer to understanding the overall regulation.

D. Specific Background Information

There are many types of growth factors, growth factor binding proteins, and proteoglycans. The focus for this text will be on insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3) and heparan sulfate proteoglycan (HSPG) secreted by bovine aortic endothelial cells (BAE).

IGF-1 is a polypeptide consisting of 70 amino acids with a molecular weight of 7000 and an overall positive charge\(^34\), (Figure 1-10). IGF-I originates primarily in the liver although many cells transiently secrete the growth factor\(^6\). IGF-1 stimulates cell growth and division, glucose and amino acid uptake, thymidine incorporation into DNA and increases liver glycogen synthesis in microvessel and endothelial cells\(^35\). IGF-I induces chemotactic activity in bovine aortic endothelial (BAE) cells\(^36\). A major portion of secreted IGF-I from BAE cells results from uptake and subsequent release rather than de novo synthesis\(^37\). IGF-I secretion and interaction with the cell surface IGF-IR is the dominant mechanism of the autocrine actions of IGF-I\(^38\). Only a quarter of the total IGF-I is found unimpeded in the bloodstream with the remaining 75% of IGF-I traveling in a 150 kDa complex with IGFBP-3 and the acid-labile subunit\(^39\). IGF-I is transported via transcytosis through capillary endothelial cells and IGFBP-3 facilitates the transport\(^40\).

IGFBP-3 is one of six known IGF binding proteins\(^39\). It has an overall positive charge\(^41\) and can bind and modulate the actions of IGF-I, (Figure 1-11). IGFBP-3 is believed to inactivate IGF-I and to act as a carrier to tissues\(^42\). IGF-I stimulation has been
IGFBP-3 blocks the binding of IGF-I to IGF-IR in porcine aortic endothelial cells. IGFBP-3 appears to exhibit a higher affinity for IGF-I than the endothelial type IGF-IR. It has been found that IGFBP-3, cultured from bovine endothelial cells, stimulate the metabolic processes of AIB and glucose uptake in cultured microvessel endothelial cells. IGFBP-3 in solution can act as a reservoir releasing continuously low amounts of IGF-I and thereby creating a steady state situation of receptor occupancy, which can act as a better mitogenic stimulus than a temporary large bolus of free IGF-I.

IGF-I has a higher affinity for circulating IGFBP-3 than for surface bound IGFBP-3 in human fetal fibroblasts (GM10) and porcine smooth muscle cells. IGFBP-3 is more effective as an inhibitor of IGF-I surface binding than IGFBP-1 or IGFBP-2. It has been shown that added IGF-I can prevent surface binding of IGFBP-3 in Sertoli cells. The increasing effect of IGF-I on IGFBP-3 abundance in cell medium is a direct result of IGF-I interaction with IGFBP-3. It is not clear if IGFBP-3 binding to specific cell surfaces is due to specific receptor-mediated events or due to cell surface HSPG interactions. Proteoglycans may interact directly with IGFBP-3 by either acting upon IGF-I/IGFBP-3 complex or the IGFBP-3/acid-labile subunit. IGFBP-3 and IGFBP-5 have the highest heparin affinity of all of the IGFBP family with a putative heparin-binding domain near the C-terminus. IGFBP-3 specifically binds to both cell surface
and the extracellular matrix (ECM) of cultured bovine periaortic and bovine pulmonary artery endothelial cell monolayers with heparin and heparan sulfate competing for binding of IGFBP-3 to the monolayer\(^{54}\). Heparin, which can release proteins bound to HSPG, by competition, has been shown to increase medium concentration of IGFBP-3 and decreases surface binding of IGFBP-3\(^{55}\). IGFBP-3 binding to the cell surface can also be prevented by the addition of IGF-I peptides which suggests a conformational change in IGFBP-3 that may result from binding\(^{48,49}\). IGFBP-3 can prevent IGF-I-induced receptor down-regulation, a process that renders cells immune to further stimulation by IGF-I\(^{56}\).

Endothelial cells cultured from fetal bovine pulmonary arteries produce a basement membrane HSPG that is a known potent inhibitor of smooth muscle cell proliferation\(^{39}\) and may be an inhibitor for endothelial cells. Typical concentration of
heparan sulfate proteoglycans on the cell surface as measured in various cell culture systems are in the range of $10^5$ - $10^6$ molecules/cell$^{57}$. Heparin, which can release proteins bound to HSPG, can inhibit formation of IGF-I/IGFBP-3 complex thus making IGF-I available to bind to the receptor$^{54}$. HSPG interrupts the normal operation of the IGF-I/IGFBP-3 complex either affecting the complex directly or acting upon the IGFBP-3/acid-labile subunit$^{53}$, thereby becoming a regulator of IGF-I stimulation in its own right. IGF-I stimulates growth of all classes of GAG containing proteoglycan in pulmonary artery cells (large vessel) and preferentially stimulates heparan sulfate containing proteoglycan in microvessel endothelial cells$^{28}$.

E. Organization

The overall objective of this research is to examine on how local extracellular factors (HSPG and IGFBP-3) impact IGF-1 binding and subsequent stimulation. Two questions are posed:

1) Do HSPG regulate IGF-1 binding and stimulation through the regulation of IGFBP-3?

2) Do vascular endothelial cell secreted HSPG expedite or impede IGF-I/IGF-IR binding?

Diagrams are shown in Figure 1-12. Figure 1-13 shows binding reactions that are possible within the bovine aortic endothelial cell. The system has many parameters and competing rates that make modeling a complicated task, (Tables 5-3, 5-5,5-7,5-9)

The text is designed in such a manner that one can read through the beginning chapter to establish base knowledge of growth factors, growth factor binding proteins and proteoglycans with a heavy emphasis on IGF-I, IGFBP-3 and HSPG in BAE cells. Chapter II focuses on materials and methods used in the study. Chapter III introduces the cell-free assays used to study the complex interactions of IGF-I, IGFBP-3 and HSPG. Chapter IV explores cell binding studies of the BAE system in question. Chapter V draws all of the previous chapters together by combining the past and present knowledge, assumptions and quantitative data to introduce a mathematical model of the IGF-1/BAE system. Chapter VI discusses the conclusions and presents ideas on future extensions of this work.
Figure 1-12 Schematic illustration depicting research questions. 1: Do HSPG regulate IGF-I binding and stimulation through the regulation of IGFBP-3? 2: Do bovine aortic endothelial cell-secreted HSPG facilitate or hinder binding of IGF-I to IGFIR?
Figure 1-13  Possible binding reactions in the tri-component system involving IGF-I, IGFBP-3 and HSPG.